

SOP: SP045

Operation of GC

Materials and Reagents:

1. Prepared samples (note 1)
2. GC needle
3. Acetone HPLC grade
4. Chloroform HPLC grade

Protocol:

1. ____ Sign up for GC located in C323.
2. ____ Turn on the Hydrogen tank and the breathing air tank and leave on for about 15 minutes before using the GC (note 2).
3. ____ Set initial temperature to 60°F and select ENTER, check if the igniter is lit (note 3).
4. ____ If the igniter is not lit, let the GC sit for 15 minutes to allow the gases to enter all lines.
5. ____ Light the igniter by holding the FID igniter button and put a flame (not matches) to the igniter.
6. ____ Gently blow across the top of the igniter (note 4).
7. ____ Check the GC program (note 5) and burn the GC column by either a) pressing start or b) set oven temp to 260°F, press enter, and let the GC sit for 20 minutes then return the temperature to 60°F.
8. ____ Set up the computer by selecting AP CHEMSTATION (APG-top). If the computer is on the DOS screen, go to C:>WIN to get to Windows.
9. ____ Select START '5890A' (model #).
10. ____ Under METHODS select LOAD and load SP2381s' then ENTER.
11. ____ After the temperature returns to 60°F, perform a column comp by pressing COLUMN COMP1, A and enter (note 6).
12. ____ Select RUN CONTROL then SAMPLE INFO.
13. ____ Under file name put in the date. Set the counter to 1 and give the sample a name (note 7).
14. ____ Make sure the ISTD amount is 10 and select OK then ENTER.
15. ____ After the column comp, record in the GC log book the signal and pressure.
16. ____ To run the neutral sugar standard, wait until the red light on the GC is off and the computer says it's ready.
17. ____ Resuspend the neutral sugar standard in 100-300µl of HPLC grade Chloroform.
18. ____ Rinse out the GC needle several times with HPLC grade Acetone.
19. ____ Tighten the syringe top and pull the plunger to 1/2µl. This will create an air bubble.
20. ____ Place the needle in the neutral sugar standard and draw the plunger up until the bottom of the air bubble is at 1µl (note 8).

21. ____ Inject the neutral sugar sample and select START on the GC.
22. ____ Rinse the GC needle with HPLC grade Acetone a few times.
23. ____ After neutral sugar sample has run, check to see if the neutral sugar is a suitable standard (note 9).
24. ____ To check the standard, select DATA ANALYSIS then MAIN SCREEN.
25. ____ Adjust axes by selecting GRAPHICS then SCALE AXES then adjust the Y axis.
26. ____ Select OK/ENTER.
27. ____ Examine the areas on the report (note 10).
28. ____ Zoom in on one peak, does it have a gradual slope up? (note 11).
29. ____ After the neutral sugar sample has been checked, re-calibrate the sugar standard.
30. ____ To recalibrate, select REPORTS and EDIT CALIBRATION TABLE.
31. ____ Reset retention values to match the retention times of the sugar standard (first column in the table).
32. ____ Double check the sugar standard settings. All the sugars should be set at 25µg except for the scyllo-inositol which should be set at 10µg.
33. ____ After re-setting the values select OK and ENTER.
34. ____ Select INTEGRATION and then INTEGRATE (note 12).
35. ____ Select REPORTS then PREP/CALIB/RECALIBRATE.
36. ____ Select REPLACE (on the right side of the table) then OK and YES to be saved.
37. ____ Select REPORTS then SPECIFY REPORTS.
38. ____ Select to print the chromatograph and the report and send the chromatograph and report to printer.
39. ____ Select REPORTS and PRINT REPORT.
40. ____ Prepare to inject another sample by selecting FILE then RETURN TO TOP.
41. ____ Select RUN CONTROL then SAMPLE INFO and change the sample name.
42. ____ When the temperature returns to 60°C, inject the sample as in step 16-22 (note 13).
43. ____ After a sample is run, if peaks are visible but not labeled (if not skip to step 46) select DATA ANALYSIS then MAIN SCREEN.
44. ____ Select INTEGRATION then INTEGRATION EVENTS and adjust the area reject (note 14).
45. ____ Select INTEGRATION then INTEGRATE. Adjust area reject as necessary.

46. _____ After all samples are run, set the initial temperature down to 35°C and turn off the Hydrogen and breathing air. Leave the GC machine and the Helium gas turned on.
47. _____ Record in the log book how many samples were run and if there were any problems.
48. _____ Close the computer programs and put the computer into sleep mode.

Notes:

1. See SOP: SP022 for preparing Alditol Acetate
2. The Helium and GC should be left on at all times as to not damage the column. Make sure there is a sufficient amount in all three tanks to run samples, if not then change the appropriate tank and label it empty.
3. To see if the igniter is lit, press SIGNAL 1 if the number is below zero it is not lit.
4. A very faint pop will sound when the GC has been lit. Also the signal will go from below zero to above zero. The signal will range depending on the age of the column and if regular maintenance has been performed on other key components of the GC. If the signal is >35 do not use.
5. The GC program should be: initial temp: 60°F, initial time: 1 min, rate: 30°/min, final temp 170°F, final time: 0, rate A 5.0°/min, final time 15 min, rate B final temp: 260°F, final time: 0. The column needs to burn off any residual material from previous runs. Both of these choices essentially do the same thing. Choice A will take 40 minutes (this runs the GC program) while choice B will take about 25 minutes
6. This run is essentially a blank run that will allow the computer to subtract any background from sample runs. All runs on the GC take about 40 minutes to complete.
7. The first sample name will be 'neutral sugar'. If this is not changed between runs it is okay because the run will automatically be saved under the file name.
8. The column has a maximum volume of 1µl. Do not attempt to load more.
9. The sugar standard must be consistent because subsequent runs are calibrated against this standard. If there is a lot of variability in the values, this will affect the values of future runs
10. The area for all the sugars should be roughly 2.5 times greater than the area for the scyllo-inositol peak.
11. This gradual slope up is called fronting and means that the column is overloaded. When this is seen it is best to dilute the sugar sample (add another 100µl of chloroform) and re-inject the sample. If this is done resuspend the rest of the samples in the same amount of chloroform.
12. Integrating the new values should re-draw the chromatograph with the correct retention times. Double check to make sure all the retention times are correct.
13. All subsequent samples are to be injected in this manner. After each run the chromatograph and report should automatically print
14. The smaller the number for the area reject the more sensitive the chromatograph (meaning that more peaks will be labeled). Usually the area reject is set at 15,000, but if it needs to be adjusted try 10,000 then 5,000 and so on.